Expression of heat shock protein 20 inversely correlated with tumor progression in patients with ovarian cancer

Naian Qiao[1*], Yanhui Zhu[2*], Haiying Li[3], Zhongyu Qu[4], Zhichun Xiao[2]

[1]Department of Radiotherapy, Shandong University Qilu Hospital, Jinan
[2]Medical Informatics Center, Peking University, Beijing
[3]Division of Ultrasonography, Shandong University Qilu Hospital, Jinan
[4]Division of Ultrasonography, Shandong Provincial Hospital, Jinan (China)

Summary

Objective: To investigate a possible correlation between expression levels of heat shock protein 20 (HSP20) and tumor progression in patients with ovarian cancer. *Materials and Methods:* The study included 34 patients with ovarian cancer who were to undergo surgery, seven patients with ovarian carcinoid tumors, and five patients with normal ovaries as a control group. Ovarian tissues were obtained from patients by surgical resection and then analyzed by western blot. *Results:* Expression levels of HSP20 were inversely correlated with the grade of malignancy. *Conclusion:* The present findings suggest that HSP20 may play a protective role against the progression of ovarian cancer. Thus, HSP20 may represent a new target for the prediction and treatment of ovarian cancer.

Key words: HSP20; Ovarian cancer; Tumor progression.

Introduction

Heat shock proteins (HSPs) are a subset of the molecular chaperones; they are best known for their rapid and abundant induction by stress. HSPs, classified by their molecular weight, are highly expressed in many malignant tumors, including ovarian cancer. Most HSPs seem to play a role in many aspects of tumor progression and response to therapy, probably due to their antiapoptotic properties [1, 2]. Previous studies have indicated that HSP27, in addition to its typical function as a chaperone, also plays fundamental roles in maintaining the intracellular redox potential and in stabilization of the cytoskeleton [3, 4]. High expression of HSP27, induced by chronic cellular stress, may lead to the suppression of apoptosis; thus, HSP27 may facilitate malignant transformation [5].

In previous studies, the authors showed that HSP20, a member of the small HSP family, has an antiapoptotic effect on cardiomyocytes [6,7]. Therefore, they hypothesized that HSP20 would also have an antiapoptotic effect on ovarian cancer cells. In the present study, the authors investigated the relationship between HSP20 expression levels and ovarian cancer progression by comparing HSP20 expression levels in specimens of ovarian cancer, ovarian carcinoid, and normal ovaries.

Materials and Methods

Subjects

The Ethics Committee of Shandong University Qilu Hospital approved the research protocol. Informed consent was obtained from each of the patients and control participants.

A total of 41 women who were hospitalized for a suspected ovarian tumor from February 2011 to January 2013 and who intended to undergo surgical intervention were randomly selected as study subjects. The demographics and clinical characteristics of the study population are indicated in Table 1. Of these, 34 women were diagnosed with ovarian cancer, seven were diagnosed with ovarian carcinoid tumors, and five were diagnosed with normal ovaries. Finally, samples of healthy ovarian tissue from five age-matched women who had died in traffic accidents were used for the healthy control group. All of the participants were Asian Chinese women.

Women with ovarian cancer and ovarian carcinoid were excluded if they had received hormone therapy or chemotherapy or if their condition occurred in combination with other malignancies. After screening, the ovarian cancer group included 34 patients: six (18%) International Federation of Gynecology and Obstetrics (FIGO) Stage I cases, ten (29%) FIGO Stage II cases, 12 (35%) FIGO Stage III cases, and six (18%) FIGO Stage IV cases. The cancers had different histological types, as follows: serous papillary carcinoma ($n = 24$), mucinous carcinoma ($n = 4$), clear cell carcinoma ($n = 3$), endometrioid carcinoma ($n = 2$), and mixed cystadenocarcinoma ($n = 1$). Another seven women with benign ovarian carcinoid were recruited for the ovarian carcinoid group. The ovarian carcinoid patients had different histological types: serous cystadenoma ($n = 2$), mucinous cystadenoma ($n = 1$), mixed cystadenoma ($n = 1$), and simple ovarian cyst ($n = 2$).

Surgical specimens

Ovarian tissues were obtained from patients by surgical resection at the Department of Obstetrics and Gynecology, Shandong University Qilu Hospital. The resected tissue was snap-frozen in liquid nitrogen and stored at −80°C until used for Western blot analysis.

[*] *Contributed equally and should be considered co-first authors.*

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Table 1. — Demographics and clinical characteristics of the study population.

Characteristics	Ovarian Cancer Stage I (n = 6)	Ovarian Cancer Stage II (n = 10)	Ovarian Cancer Stage III (n = 12)	Ovarian Cancer Stage IV (n = 6)	Normal (n = 5)	Ovarian carcinoid (n = 7)
Age, y						
Mean (SD)	53.2 (1.9)	54.5 (1.8)	57.5 (2.1)	59.2 (1.5)	52.9 (2.4)	51.7 (3.0)
Range	46–58	46–59	51–62	54–63	45–58	42–60
Age distribution, n (%)						
≤ 55 years	3 (50%)	4 (40%)	5 (42%)	2 (33%)	4 (57%)	3 (60%)
> 55 years	3 (50%)	6 (60%)	7 (58%)	4 (67%)	3 (43%)	2 (40%)
Race, n (%)						
Asian Chinese	6 (100%)	10 (100%)	12 (100%)	6 (100%)	7 (100%)	5 (100%)
Ovarian cancer stage						
Stage I	6 (18%)					
Stage II		10 (29%)				
Stage III			12 (35%)			
Stage IV				6 (18%)		
Histology						
Serous	5 (15%)	8 (24%)	7 (21%)	4 (12 %)		
Mucinous	1 (3%)	1 (3%)	2 (6%)	0		
Clear cell	0	0	1 (3%)	2 (6%)		
Endometrioid	0	1 (3%)	1 (3%)	0		
Mixed cystadenocarcinoma	0	0	1 (3%)	0		

Western blot analysis

Snap-frozen samples were homogenized and sonicated in lysis buffer containing 20 mmol/L Tris-HCl pH 7.4, 1% Trion X-100, 150 mmol/L sodium chloride, one mmol/L ethylenediaminetetraacetic acid (EDTA), 2.5 mmol/L sodium pyrophosphate, one mmol/L sodium fluoride, one mmol/L sodium orthovanadate, and 0.1 mmol/L phenylmethylsulfonyl fluoride. Aliquots were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes and incubated with primary polyclonal anti-HSP20 antibodies at 4°C overnight. Bound antibodies were detected with a secondary antibody conjugated to horseradish peroxidase, visualized by use of an enhanced chemiluminescence kit, and exposed to X-ray film for the appropriate time [6, 7]. Protein band intensities were determined by integrating the optical density over the band area (band volume) with NIH imaging software. HSP20 levels were normalized to those of b-actin.

Statistical analysis

Data were expressed as means ± standard deviation (SD). Differences were analyzed for significance by one-way repeated-measures ANOVA and further analyzed with the Newman–Keuls test for multiple comparisons between treatment groups. The results were considered significant at $p < 0.05$. GraphPad Prism version 4.0 for Windows was used for the analysis.

Results

Expression of HSP20

Western blot images of HSP20 expression in six representative patients with normal ovaries; ovarian carcinoid; and ovarian cancer Stages I, II, III, and IV are shown in Figure 1. There were decreased levels of HSP20 expression in the tumor tissues, a trend toward decreased expression levels of HSP20 in tumor tissues was observed.

Comparison of HSP20 levels in different stages of ovarian cancer

HSP20 levels in tissue samples of different stages of ovarian cancer were compared to HSP20 levels in ovarian carcinoid tumors and normal ovaries. There were significant differences in HSP20 levels with respect to tumor progression ($p < 0.05$, Figure 2). There were also significant differences in HSP20 levels when any two ovarian cancer Stages (I, II, III, and IV) were compared ($p < 0.05$ for all comparisons). The present authors observed a trend toward decreased expression levels of HSP20 in tumor tissues that were inversely correlated with increasing cancer stage.

Discussion

The present investigation of the association between HSP20 expression levels and tumor progression in patients with ovarian cancer revealed a trend toward decreased HSP20 expression levels in tumor tissues. The results suggest that levels of HSP20 in tumor tissues were attenuated in parallel with ovarian cancer progression; thus, HSP20 expression levels were inversely related to the grade of malignancy. To the best of the authors' knowledge, this is the first report of a significant association between HSP20 levels and the progression of ovarian cancer.

The idea that HSPs may protect against disease is not unprecedented In a previous study, the present authors demonstrated that overexpression of HSP20 in rat hearts is protective against ischemia/reperfusion; the protective effect is related to a reduction in the necrosis and apoptosis of ventricular cardiomyocytes both in vitro and in vivo [6, 7]. Another previous study reported that HSP60 mRNA lev-

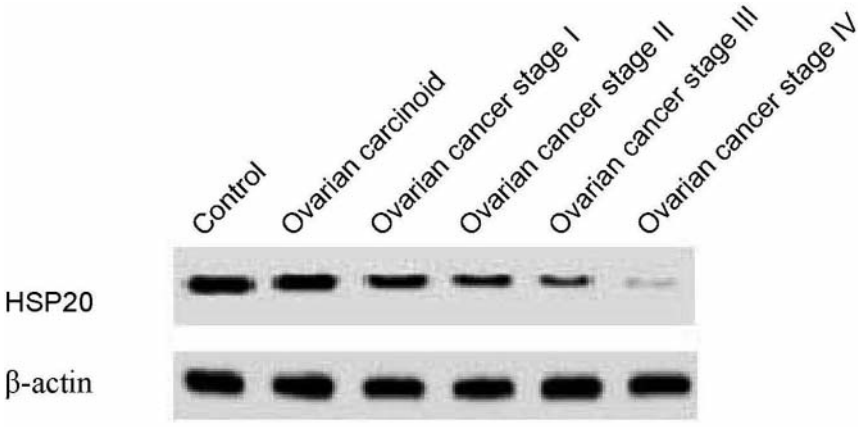

Figure 1. — Western blot showing HSP20 levels in six representative specimens: four ovarian cancer, one ovarian carcinoid, and one control. Protein extracts were analyzed with antibodies against HSP20 and β-actin.

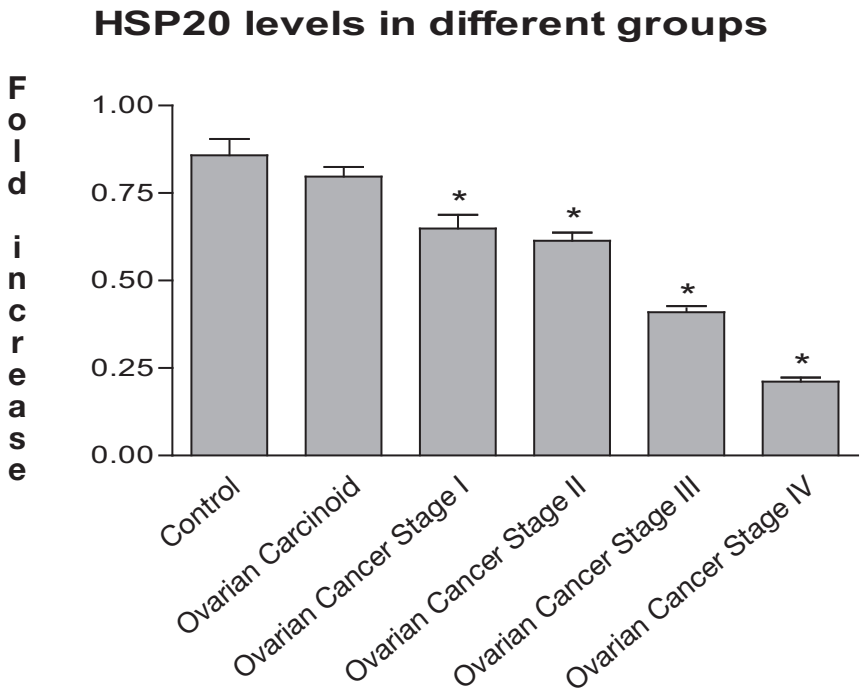

Figure 2. — HSP20 levels in ovarian cancer and control specimens. Protein extracts from 34 ovarian cancer specimens, seven ovarian carcinoid tumor specimens, and five control specimens were analyzed with antibodies against HSP20 and b-actin. Signal intensities on X-ray film were quantified with NIH imaging software. Histograms show quantitative representations of HSP20 levels after normalization to b-actin levels. Values on the vertical axis represent the mean ± standard error of the mean of independent experiments. *$p < 0.05$ compared to control tissue samples, p was also < 0.05 when any two ovarian cancer Stages (I, II, III, and IV) were compared.

els are a valuable prognostic factor for epithelial ovarian cancer. Variations in expression levels were not due to amplification of this gene [8]. Moreover, Olejek *et al.* reported that the mean concentration of anti-Hsp27 antibodies in a group of patients with ovarian carcinoma was significantly higher than in the control group. Their analysis of the association between anti-Hsp27 antibodies and the stage of clinical progression revealed that the concentration of anti-Hsp27 antibodies was higher in less advanced ovarian carcinoma specimens [9].

Conclusion

The present results strongly suggest that expression levels of HSP20 decrease with tumor progression in ovarian cancer patients; thus, HSP20 could have a suppressive effect on the progression of ovarian cancer. The present authors are currently conducting studies to investigate the underlying mechanism for this effect and to optimize the detection of anti-HSP20 antibodies in serum for the early identification of ovarian cancer.

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Address reprint requests to:
YANHUI ZHU, M.D.
Peking Univerisity Medical Informatics,
38 Xueyuan road, Beijing 100191 (China)
e-mail: gzyh@hsc.pku.edu.cn